

The Candidate Blood-stage Malaria Vaccine P27A Induces a Robust Humoral Response in a Fast Track to the Field Phase 1 Trial in Exposed and Nonexposed Volunteers

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Background. P27A is an unstructured 104mer synthetic peptide from *Plasmodium falciparum* trophozoite exported protein 1 (TEX1), the target of human antibodies inhibiting parasite growth. The present project aimed at evaluating the safety and immunogenicity of P27A peptide vaccine in malaria-nonexposed European and malaria-exposed African adults.

Methods. This study was designed as a staggered, fast-track, randomized, antigen and adjuvant dose-finding, multicenter phase 1a/1b trial, conducted in Switzerland and Tanzania. P27A antigen (10 or 50 µg), adjuvanted with Alhydrogel or glucopyranosil lipid adjuvant stable emulsion (GLA-SE; 2.5 or 5 µg), or control rabies vaccine (Verorab) were administered intramuscularly to 16 malaria-nonexposed and 40 malaria-exposed subjects on days 0, 28, and 56. Local and systemic adverse events (AEs) as well as humoral and cellular immune responses were assessed after each injection and during the 34-week follow-up.

Results. Most AEs were mild to moderate and resolved completely within 48 hours. Systemic AEs were more frequent in the formulation with alum as compared to GLA-SE, whereas local AEs were more frequent after GLA-SE. No serious AEs occurred. Supported by a mixed Th1/Th2 cell-mediated immunity, P27A induced a marked specific antibody response able to recognize TEX1 in infected erythrocytes and to inhibit parasite growth through an antibody-dependent cellular inhibition mechanism. Incidence of AEs and antibody responses were significantly lower in malaria-exposed Tanzanian subjects than in nonexposed European subjects.

Conclusions. The candidate vaccine P27A was safe and induced a particularly robust immunogenic response in combination with GLA-SE. This formulation should be considered for future efficacy trials.

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Keywords. malaria; vaccine; GLA-SE; blood-stage; ADCI.

Due to increasing drug resistance against antimalarial drugs, the development of a safe and effective vaccine would be an invaluable tool in the fight against malaria, and eventually eradication of malaria [1]. Opinions are diverse on how antibodies to blood-stage antigens achieve protection: inhibition of merozoite invasion into erythrocytes, triggering of the release by monocytes of parasitostatic and parasitocidal substances via antibody-dependent cellular inhibition (ADCI), or inhibition of cytoadherence of infected red blood cells to endothelial cells [2–5]. In our search for novel vaccine candidates through genome mining, we have addressed the first 2 paradigms in a systematic manner and identified trophozoite exported

protein 1 (TEX1) (also known as PF3D7_0603400, PFF0165c, or MAL6P1.37, here referred to as *Pf27*) [6, 7]. A highly conserved segment of *Pf27* corresponding to a sequence predicted to assume a random coiled structure of 104 amino acids (peptide 27A [P27A]) was synthesized and purified. P27A was found to be highly antigenic and the target, at high prevalence, of B- and T-cell responses in individuals living in malaria-endemic areas [7, 8]. The antibodies developed by protected individuals were predominantly cytophilic immunoglobulin G1 (IgG1) and immunoglobulin G3 (IgG3) able to inhibit parasite growth in an antibody-dependent cellular fashion (ADCI). Interestingly, in a parallel analysis of the response to a recombinant candidate vaccine including P27A, the antibody response of African volunteers was strongly associated with clinical protection (G. Corradin et al, submitted manuscript). Although the soluble factors, including tumor necrosis factor alpha (TNF-α), involved in ADCI are still largely uncharacterized, ADCI is the result of the overall functional effect of antibodies and monocyte collaboration on in vitro parasite growth [9]. Recent data

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suggest a potential role of the ADCI assay as a correlate of protection.

The objective of this trial was to assess the safety and immunogenicity of a synthetic peptide vaccine candidate based on the P27A fragment of *Pf27*, with the aim to induce a protective antibody response that may complement immune responses induced by antigen(s) from the preerythrocytic stage in a multicomponent vaccine.

METHODS

Trial Design and Study Participants

The study was designed as a staggered, randomized, antigen and adjuvant dose-finding, multicenter phase 1a/1b clinical trial using the fast-track strategy set by the European Vaccine Initiative and its partners to accelerate malaria vaccine clinical development. Study was conducted in Switzerland for phase 1a and in Tanzania for phase 1b.

The phase 1a (Lausanne, Switzerland [CH]) was designed as a single-center, staggered randomized, volunteer- and laboratory-blinded trial. Healthy adult men and women aged 18–45 years were eligible to participate when human immunodeficiency virus (HIV) negative, without known exposure to malaria, and P27A or parasite antibody negative by enzyme-linked immunosorbent assay (ELISA). Phase 1b (Bagamoyo, Tanzania [TZ]) was designed as a single-center, staggered randomized, double-blind, controlled trial. Healthy adult volunteers aged 18–45 years were eligible to participate when HIV negative, having lived in areas of Tanzania with minimal malaria transmission (urban Dar es Salaam). As contraception is not always practiced by women of childbearing age in the study population, female subjects were not included. Participants were enrolled if they met the inclusion criteria (see [Supplementary Materials](#) for details) and, for the nonexposed group, were not pregnant or lactating. The phase 1a volunteers were allocated (on a 1:1 basis) into 2 groups of 8 volunteers each, who were injected intramuscularly with 50 µg of the P27A antigen adjuvanted with Alhydrogel (group CH-Alum/50) or 2.5 µg glucopyranosil lipid adjuvant stable emulsion (GLA-SE) (group CH-GLA2.5/50). The phase 1b volunteers were randomized and allocated (on a 4:1 basis) in a dose-escalating manner to 4 cohorts. Each cohort included 10 subjects, 8 of whom were injected intramuscularly with 50 µg P27A and Alhydrogel (group TZ-Alum/50), with 10 µg P27A and 2.5 µg GLA-SE (group TZ-GLA2.5/10), with 50 µg P27A and 2.5 µg GLA-SE (group TZ-GLA2.5/50), and with 50 µg P27A and 5 µg GLA-SE (group TZ-GLA5/50). Two subjects per cohort were injected with the rabies vaccine Verorab as control (group TZ-Ver). Injections were performed at days 0, 28, and 56 with a follow-up of 6 months ([Figure 1](#)).

The transition phase from the European to African trial population started after completion of the first injection series of

each group sequentially in the European site and after evaluation of the safety data by an independent data and safety monitoring board (DSMB). A 2-week stagger was left to ensure safety and reactogenicity evaluation prior to transition to the next higher P27A dosage or change from Alhydrogel to GLA-SE in the same site, and a 4-week stagger for transition from European phase 1a to African phase 1b ([Figure 1](#)). Further information on study design, including the clinical trial protocol, is provided in the [Supplementary Materials](#).

Study Vaccines and Procedures

The investigational vaccine antigen and adjuvants were produced under Good Manufacturing Practice (GMP) constraints according to relevant national regulations. The bulk P27A 104-residue synthetic peptide was manufactured by Almac (Craigavon, United Kingdom) and further diluted, filled in monodose vials by Nova Laboratories Ltd (Leicester, United Kingdom). The bulk Alhydrogel was manufactured by Brenntag (Frederikssund, Denmark) and diluted with water for injection, filled into vials by Nova Laboratories Ltd. P27A and Alhydrogel vials have been labeled, released, and shipped to the clinical sites by Nova Laboratories Ltd. The GLA-SE and the GMP EM060-SE used as diluent were manufactured by the Infectious Disease Research Institute (Seattle, Washington). The GLA-SE and SE diluent were labeled, released, and shipped by Output Pharma GmbH (Aachen, Germany). The P27A vaccine antigen was formulated with 1 of the 2 adjuvants at each site pharmacy prior to the injection.

Primary Outcome

Safety and tolerability of the vaccine were assessed based on the number and intensity of solicited and unsolicited adverse events (AEs). The safety profile included local and systemic AEs as well as the biological safety tests, based on clinically significant changes of the baseline value of the main biological criteria.

Further information on secondary and exploratory outcomes (immunogenicity) as well as statistical analysis is provided in the [Supplementary Materials](#).

RESULTS

Participant clinical characteristics are provided in the [Supplementary Materials](#) and [Supplementary Table 1](#).

Safety and Reactogenicity

Local Reactogenicity

Local AEs from day 0 to day 7 are summarized in [Figure 2A](#), and are presented as the cumulative number of events occurring in each vaccination group. Local reactogenicity was reported by 100% and 82.5% of volunteers in phase 1a and 1b, respectively, and was mostly mild to moderate and self-limited. Local AE were more frequent after GLA-SE. Detailed description of local reactogenicity can be found in the [Supplementary Materials](#).

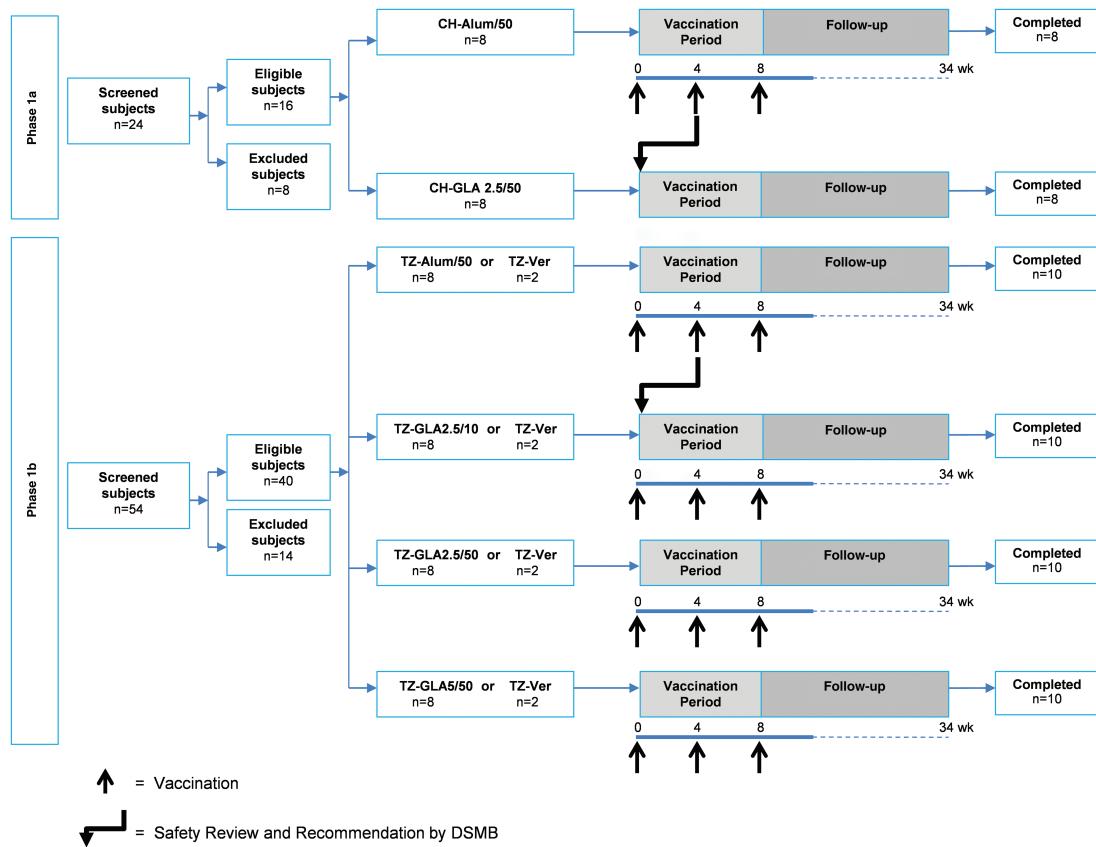


Figure 1. Trial profile, injection schedule, and safety follow-up. Abbreviations: CH, Lausanne, Switzerland; DSBM, data and safety monitoring board; GLA, glucopyranosil lipid adjuvant; TZ, Bagamoyo, Tanzania; Ver, Verorab.

Systemic Reactogenicity

Systemic AEs (solicited and unsolicited) from day 0 to day 7 are summarized in Figure 2B, and are presented as the cumulative number of events in each vaccination group, and described below in the text as number of AEs per injections.

In phase 1a, systemic AEs occurred in 39 of 48 injections (81.3%), most of them being considered as unrelated to the vaccination (76/125 events [60.8%]). The most frequent solicited systemic AEs were tiredness (reported after 11/24 injections [48.5%] for group CH-Alum/50 and 10/24 injections [41.7%] for group CH-GLA2.5/50) and headache (7/24 injections [29.2%] for group CH-Alum/50 and 7/24 injections [29.2%] for group CH-GLA2.5/50). Fever was not reported during the evaluation period. Up to 1 month after the third injection, a total of 53 unsolicited systemic AEs following 29 of 48 injections (60.4%) were reported from 15 subjects (8 subjects for group CH-Alum/50 and 7 subjects for group CH-GLA2.5/50) and resolved without sequelae. Forty-four were grade 1 AEs (3 related to vaccination), 5 were grade 2 (1 related), and 3 reached grade 3 with no relationship with vaccination. One was left ungraded. There were no significant abnormal vital signs and no relevant changes in physical examination. No clinically relevant variations in blood cell counts and biochemistry analysis were recorded.

In the phase 1b population, systemic AEs occurred after 25 of 120 injections (19/40 subjects) (47.5%) for a total of 39 events, mostly reported as related to vaccination (25/39 [64.1%]). The most frequent solicited systemic AEs were headache (reported after 3/24 injections [12.5%] for group TZ-Alum/50 and 1/24 injections [4.2%] for group TZ-GLA2.5/10, 2/24 injections [8.3%] for group TZ-GLA2.5/50, 1/24 injections [4.2%] for group TZ-GLA5/50, 1/24 injections [4.2%] for group TZ-Ver), and fatigue (2/24 injections [8.3%] for group TZ-Alum/50 and 0/24 injections [0%] for group TZ-GLA2.5/10, 2/24 injections [8.3%] for group TZ-GLA2.5/50, 1/24 injections [4.2%] for group TZ-GLA5/50, 0/24 injections [0%] for group TZ-Ver). At least 1 subject experienced systemic unsolicited AE during each vaccination; however, no grade 3 AE was reported. Fever was not reported during the evaluation period. There were no significant abnormal vital signs and no relevant changes in physical examination. No significant variations in blood cell counts and biochemistry analysis were recorded.

Comparison Between Adjuvants or Populations

Statistical differences in frequency of AEs between CH and TZ groups having received the same vaccine formulations are shown in Figure 2 and Supplementary Tables 2 and 3 (Fisher exact test). With regard to adjuvants, cumulating all local AEs

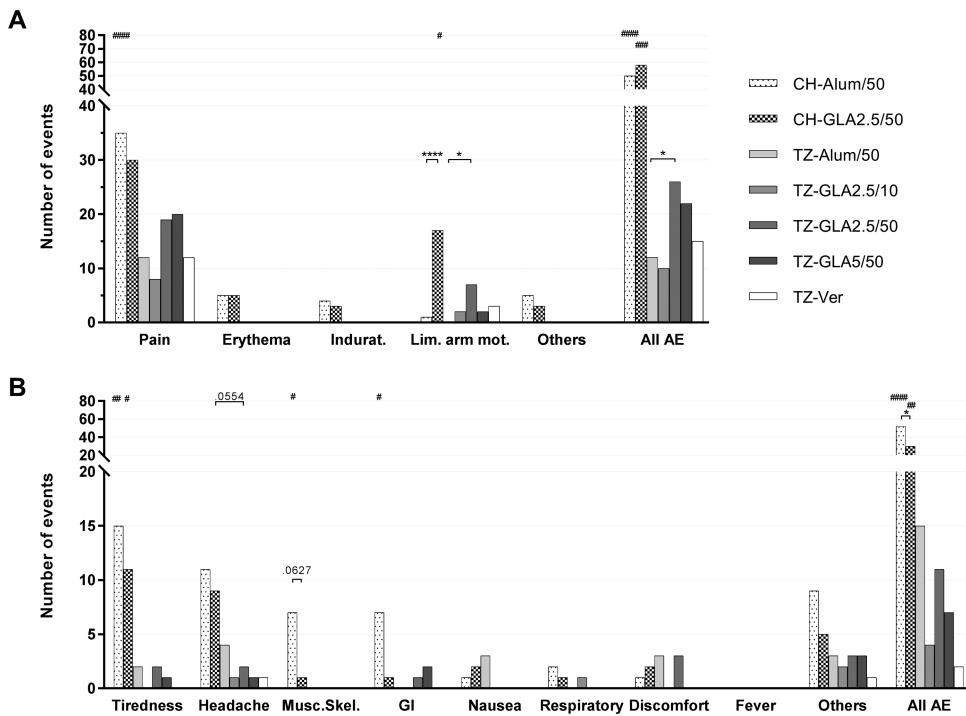


Figure 2. Local and systemic reactogenicity. Cumulative number of local (*A*) and systemic (*B*) adverse events (solicited and unsolicited) observed from day 0 to day 7 after each vaccination in each group are indicated. For each parameter, 72 reports were done per group, 8 subjects per group, 3 injections, and 3 time points per injection (60 minutes postvaccination, day 1 ± 12 hours, and day 7 ± 24 hours). Comparisons of numbers of events between the 4 groups that received Alum/50 or glucopyranosil lipid adjuvant (GLA) 2.5/50 were done using Fisher exact contingency tests, each group taken separately; *P* values are indicated as * or # for comparisons of adjuvants (alum vs GLA-stable emulsion) or populations (Lausanne, Switzerland vs Bagamoyo, Tanzania), respectively. Abbreviations: AE, adverse event; CH, Lausanne, Switzerland; GLA, glucopyranosil lipid adjuvant; Indurat., induration; Lim. arm mot., limitation in arm motion; Musc.Skel, musculoskeletal AEs; TZ, Bagamoyo, Tanzania; Ver, Verorab. *#*P*<.05; **#*P*<.01; ***#*P*<.001; ****#*P*<.0001.

from volunteers who received the same adjuvant at the same dose of peptide, there was no significant difference between alum and GLA-SE (*P* = .066), whatever the severity of the reaction. However, limitation in arm motion was more frequently induced after GLA-SE (*P* < .0001). Systemic AEs (all) were significantly more frequent after Alum (*P* = .02), with a trend toward more musculoskeletal AEs with alum (*P* = .07). When AEs (all) were compared according to sites, CH vs TZ, we observed significantly more frequent AEs in the Swiss volunteers than in the Tanzanian volunteers both for local (all AEs, *P* < .0001; odds ratio [OR], 3.167, as well as individual AEs) and systemic AEs (all, *P* < .0001; OR, 3.429, and tiredness, headaches, musculoskeletal, and gastrointestinal AEs). Grade 3 local AEs were also more frequent in the Swiss population (*P* = .011; OR, 10.13).

Immunogenicity

IgG Antibody Responses

Nonexposed phase 1a volunteers mounted a specific anti-P27A IgG antibody response that peaked at day 84 showing median titers of 3200 (range, 200–12 800) and 51 200 (range, 3200–204 800) in groups CH-Alum/50 and CH-GLA2.5/50, respectively (Figure 3). A difference in median titers of at least

10-fold between the 2 groups at day 84 persisted at day 238 (week 34), that is, 26 weeks after the last immunization. Titers were maintained at a high level in group CH-GLA2.5/50, with median titer of 9600 (range, 1600–51 200) at day 238 while group CH-Alum/50 displayed a median titer of 400 (range, 50–800).

In the exposed phase 1b volunteers, 9 of 40 volunteers already presented a positive humoral response to P27A at day 0 according to criteria defined for the screening of the phase 1b volunteers. IgG titers at day 84 in groups TZ-Alum/50, TZ-GLA2.5/10, and TZ-GLA2.5/50 reached median titers of 4800 (range, 100–9051), 2400 (range, 800–12 800) and 6400 (range, 1600–12 800), respectively (Figure 3). These responses were in the same range as those obtained at day 84 in group CH-Alum/50. Nevertheless, group TZ-GLA2.5/50 responses persisted significantly longer than in group TZ-Alum/50 (Kruskal-Wallis *P* value of .0008, with a posttest *P* value of .0103 at day 238). Volunteers from group TZ-GLA5/50 showed even stronger responses at day 84 with median titers of 13 577 (range, 9051–25 600), levels comparable to those obtained in group CH-GLA2.5/50 of 51 200 (range, 3200–204 800) (*P* = .1290). No variations in anti-P27A IgG titers were observed in the TZ-Ver control group.

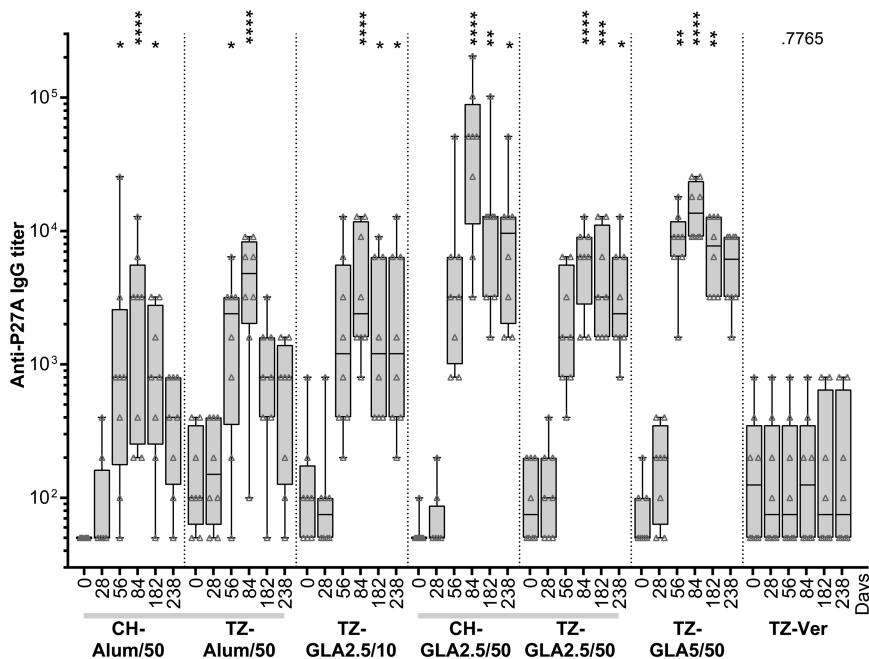


Figure 3. Anti-P27A immunoglobulin G responses. Kinetics of responses in 6 groups that received P27A formulated in Alhydrogel (Switzerland [CH]–Alum/50 and Tanzania [TZ]–Alum/50) or glucopyranosil lipid adjuvant (GLA) stable emulsion (TZ-GLA2.5/10, CH-GLA2.5/50, TZ-GLA2.5/50, and TZ-GLA5/50) or Verorab as control (TZ-Ver) are shown as box plots and whiskers (interquartile range, minimum and maximum). Comparisons intragroup were performed using Friedman test; *P* values of analysis of variance were at least $<.001$ for vaccinees; *P* values of Dunn posttest of comparison with day 0 are indicated. Abbreviations: CH, Lausanne, Switzerland; GLA, glucopyranosil lipid adjuvant; IgG, immunoglobulin G; TZ, Bagamoyo, Tanzania; Ver, Verorab. **P* < .05; ***P* < .01; ****P* < .001; *****P* < .0001.

IgG Subclasses and IgM Responses

The levels of P27A specific IgG1, immunoglobulin G2 (IgG2), IgG3, immunoglobulin G4 (IgG4), and IgM were assessed by ELISA in all phase 1a and 1b volunteers at day 84 (peak response) (Figure 4). The vaccine formulation GLA2.5/50 induced higher IgG1, IgG2, IgG4, and immunoglobulin M (IgM) responses in the nonexposed Swiss group than in the semi-immune group TZ-GLA2.5/50, but no significant difference in IgG3 levels (Figure 4B–F). P27A in Alhydrogel induced a higher IgG3 response in group TZ-Alum/50 volunteers with a median titer of 800 (range, 100–6400) than in group CH-Alum/50 volunteers (median titer of 150 [range, 12.5–400]) (Mann-Whitney *P* value of .0458) with no difference with the other subclasses. Clearly Alhydrogel failed to induce IgM in both nonexposed and exposed volunteers (group CH-Alum/50 and TZ-Alum/50) (Figure 4B).

In Vitro Parasite Growth Inhibition Assay ADCI

Eleven of 16 subjects from group CH-Alum/50 and CH-GLA2.5/50 showed an increase in the inhibitory capacity of specific IgG with vaccination, without significant difference between the 2 groups (Figure 5A). IgM and IgG3 isotypes appeared to be associated with the strongest parasite growth inhibition, although the correlation was nonsignificant (Figure 5B and 5C).

Further information on other secondary endpoints (Western blot recognition of TEX1, antibody response to parasite by indirect

fluorescence antibody test [IFAT], peripheral blood mononuclear cell [PBMC] cytokine profile) is provided in the [Supplementary Materials](#). In brief, Western blotting based on affinity-purified IgG revealed 2 bands at 165 kDa and 130 kDa specifically recognized postvaccination in 5 volunteers from the CH-Alum and GLA-SE cohorts, compatible with previous descriptions of TEX1 migration [7] ([Supplementary Figure 1A](#)). IFAT at day 84 showed a positive immunofluorescence closely associated with intraerythrocytic trophozoites ([Supplementary Figure 1B](#)). P27A vaccination induced significant responses for interferon gamma (IFN- γ), interleukin (IL) 2, IL-5, IL-10, and TNF- α starting at day 56 and still present at day 238 ([Supplementary Figure 2A–J](#)).

DISCUSSION

Blood-stage vaccines are aiming to achieve nonsterile protective immunity with hopefully a good memory response, an aspect which has been so far one of the major weakness of the most advanced, partially effective vaccine tested in a phase 3 trial, the preerythrocytic vaccine RTS,S [10]. In this respect, this paper shows that an unstructured segment from the TEX1 of *P. falciparum* appears an ideal candidate for a synthetic peptide vaccine. Indeed, P27A vaccine was not only safe, but also induced a strong specific humoral response in a formulation with GLA-SE. Alhydrogel is a classical, well-tolerated vaccine adjuvant with a good capacity to induce a robust humoral,

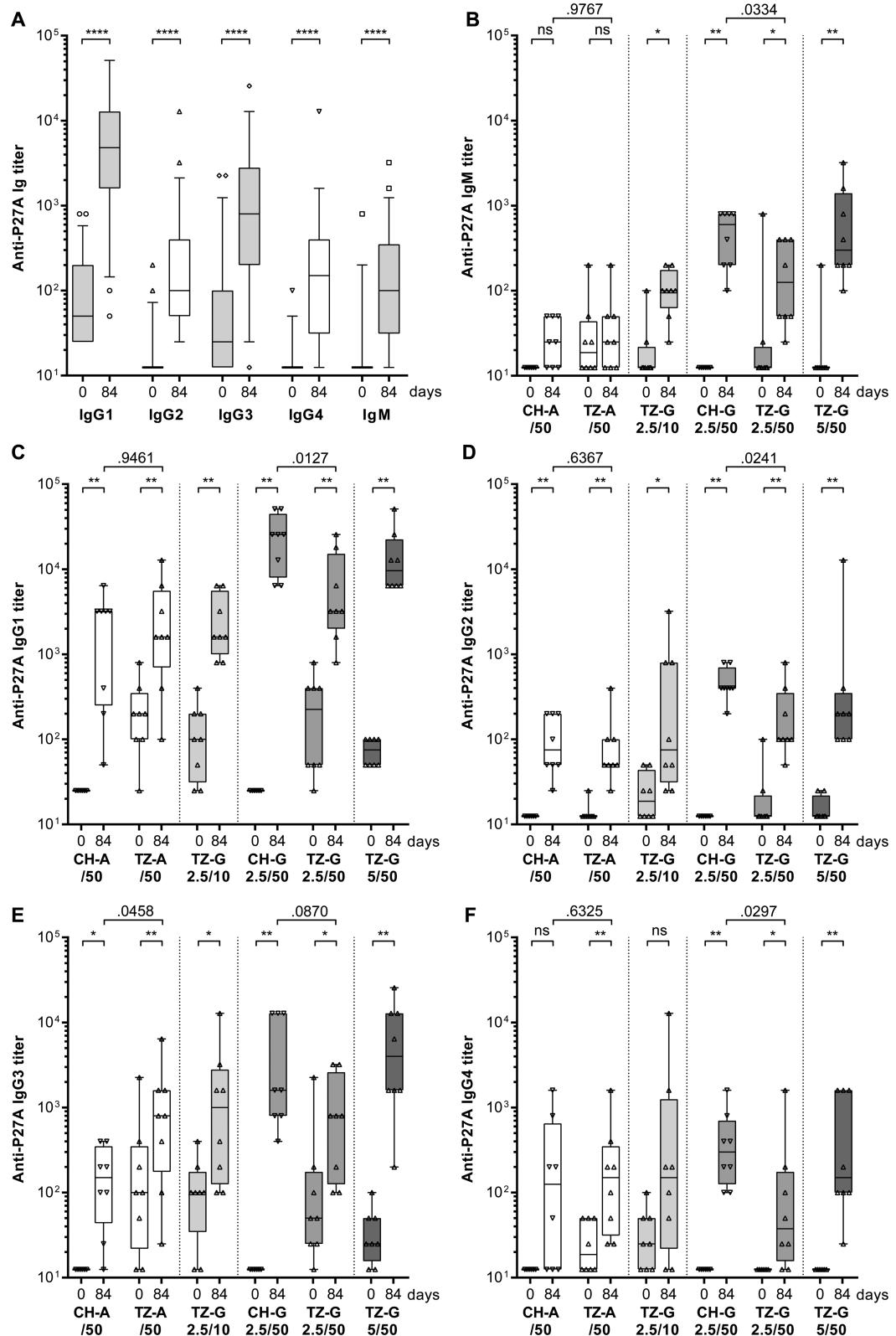


Figure 4. Anti-P27A isotypic responses. A, P27A-specific immunoglobulin G (IgG) 1, 2, 3, 4 and immunoglobulin M (IgM) titers at day 0 and day 84 in all volunteers who received P27A formulated in Alhydrogel (group Switzerland [CH]–Alum/50, Tanzania [TZ]–Alum/50) or glucopyranosil lipid adjuvant (GLA) stable emulsion (group TZ-GLA2.5/10, CH-GLA2.5/50, TZ-GLA2.5/50, and TZ-GLA5/50) as box plots and whiskers (interquartile range and 5th and 95th percentiles). Responses per group are shown for IgM (B), IgG1 (C), IgG2 (D), IgG3 (E), and IgG4 (F). Comparisons for day 84–day 0 were performed using Wilcoxon paired tests, comparisons within groups of identical formulations using Mann-Whitney tests. Abbreviations: A, alum; CH, Lausanne, Switzerland; G, glucopyranosil lipid adjuvant; IgG, immunoglobulin G; IgM, immunoglobulin M; ns, not significant; TZ, Bagamoyo, Tanzania. * $P < .05$; ** $P < .01$; *** $P < .001$; **** $P < .0001$.

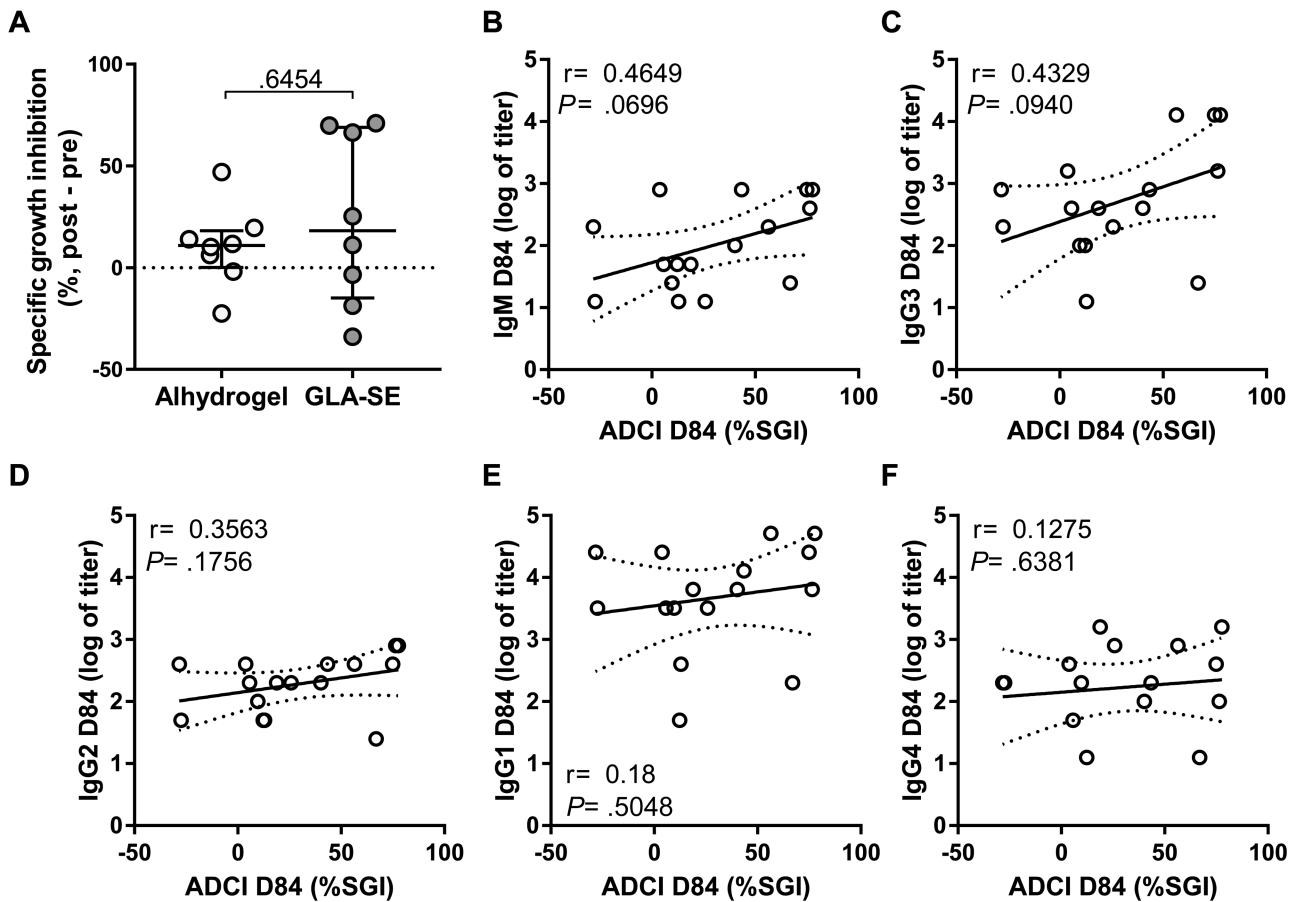


Figure 5. Antibody-dependent cellular inhibition (ADCI). Purified anti-P27A immunoglobulin from phase 1a volunteers ($n = 8$ per group) were evaluated in ADCI using a model of infection of human red blood cells by *Plasmodium falciparum* in presence of human monocytes. *A*, Results expressed as the delta post-pre (day 84–day 0) of percentage of inhibition of the infection. Lines represent medians and quartiles. A Mann-Whitney test was performed to compare adjuvant formulations (Alhydrogel or glucopyranosil lipid adjuvant stable emulsion) and the *P* value is indicated. *B–F*, Correlations between ADCI and anti-P27A immunoglobulin G isotypes at D84 with Pearson *r*, *P* value, and 95% confidence bands. Abbreviations: ADCI, antibody-dependent cellular inhibition; GLA-SE, glucopyranosil lipid adjuvant stable emulsion; IgG, immunoglobulin G; IgM, immunoglobulin M; SGI, sporozoite growth inhibition.

preferentially but not exclusively Th2 response, whereas GLA, a Toll-like receptor 4 (TLR4) agonist, is a strong T-helper 1 (Th1) cell inducer recently tested in various early phase vaccine trials in humans including tuberculosis, and malaria, among others [11–13]. In this trial, humoral response was supported by a mixed Th1/T-helper 2 (Th2) cell-mediated immunity, leading to preferentially IgG1 and IgG3 antibody subclass response. Importantly, P27A antibodies were able to recognize TEX1 in vitro in Western blot analysis as well as in vivo on the parasite itself, as shown by indirect immunofluorescence assays, and finally led to effective antibody-dependent growth inhibition of the parasite.

This study protocol was designed to spare as much time as possible in moving from phase 1a to phase 1b in endemic area. This design permitted quick achievement of the various study steps sequentially and in parallel from the phase 1a study site in Lausanne, Switzerland, to the African study site 2–4 weeks later. In <6 months, all volunteers from phase 1a and 1b had received at least their first injection of the investigational vaccine. This

fast-track design was closely supervised by a single academic sponsor, the Centre Hospitalier Universitaire Vaudois, which was in constant contact with investigators from both sites to interact efficiently with ethics review boards and regulatory authorities both in Switzerland and in Tanzania. Safety was furthermore closely reviewed by a DSMB that had the responsibility to recommend to the sponsor the sequential progression from phase 1a to phase 1b. Overall, this type of design was not only administratively light and efficient, but also cost saving. It appeared ideally suited for a fast track to the field, accelerating development toward efficacy trials. Last, but not the least, it allowed head-to-head comparison of safety and immunogenicity data in 2 different populations.

This phase 1a/1b trial showed the vaccine formulations to be safe as no vaccine-related serious AE was observed. Reactogenicity was generally good. Local and systemic AEs were of low-grade severity, both in nonexposed and in exposed populations. Interestingly, nonexposed European

volunteers reported more frequent local and systemic complaints than malaria antigen–primed Tanzanian participants. These differences may be real or, rather, related to cultural perceptions. Interestingly, formulation of P27A with Alhydrogel vs GLA-SE, a TLR4 agonist, displayed rather different AEs profiles, with significantly more frequent limitation in arm motion in GLA-SE groups for local AEs. Despite this difference, arm motion limitation was mostly self-limited, benign to moderate (grade 1 or 2) and reached grade 3 in only 1 occasion. In contrast, comparing cumulative AEs from groups receiving the same dose of antigen and adjuvant, Alhydrogel appeared more reactogenic than GLA-SE in terms of systemic AEs. These comparisons have to be taken nonetheless with caution because of the sample size. This is in agreement with a previous first-in-human phase 1 study based on a GLA-SE formulated leishmaniasis vaccine [14]. The good tolerability of GLA-SE at the dosages of 2.5 and 5 µg was confirmed in the P27A trial as, irrespective of the P27A dosage (10 or 50 µg), we did not notice any evidence of induction of systemic inflammatory reaction.

The humoral immune response, the main secondary outcome, was particularly robust in phase 1a volunteers, especially after immunization with 50 µg P27A formulated in GLA-SE 2.5 µg, leading to specific antibody titers 10-fold higher than those obtained with Alhydrogel. Comparing both populations, it appears that antibody titers in Tanzanians required a higher adjuvant dosage (GLA-SE 5 µg) to reach titers comparable to the Europeans. These findings must be confirmed with larger number of volunteers, but several factors may explain this difference. First, the fact that most Tanzanian volunteers were naturally primed against P27A as demonstrated by higher day 0 anti-P27A and anti-*P. falciparum* IgG background levels, an observation also reported by others [15], but so far not fully understood. Second, parasite infestation such as helminth infections may have contributed to a reduced response [16]. Other genetic (human leukocyte antigen) or environmental factors may have played a role also, including exposure to malaria itself [17].

Importantly, as demonstrated with sera from group CH-Alum/50 and CH-GLA2.5/50 volunteers, the P27A vaccine induced IgG antibodies able to inhibit parasite growth in ADCI assay, a test performed in blinded manner. IgG1 and IgG3 subclass responses known to exhibit cytophilic activity [6, 8] predominated. Data from this trial are in line with parallel findings that antibody response to a recombinant vaccine candidate including P27A was strongly associated with clinical protection (G. Corradin et al, submitted manuscript). Furthermore, they are also in agreement with a recent observation that ADCI activity was significantly associated with reduced risk against malaria [18]. A key outcome of this trial was the demonstration that that anti-P27A antibodies induced by formulation with Alhydrogel as well as GLA-SE were able to recognize the natural

TEX1 protein as expressed by the parasite in vivo as well as in vitro in Western blot analyses.

This humoral response was supported by a mixed Th1/Th2 cell-mediated immunity (see *Supplementary Figure 2*). Peaking between day 56 and day 84, IFN-γ, IL-2, and TNF-α responses from PBMCs were robust, both in combination with Alhydrogel as well as GLA-SE, a cytokine profile in agreement with previous vaccine trials with Alhydrogel or GLA-SE [14, 19]. IL-10 and IL-5 were also induced, at lower levels, underlining the mixed Th1/Th2 response to P27A, comparable in African and Swiss volunteers.

In summary, the candidate vaccine P27A appears to be safe and able to generate a robust antibody-specific response with parasite growth inhibitory capacity. This was particularly true when the vaccine was formulated with GLA-SE 5 µg and P27A 50 µg, the preferred formulation for the next developmental step of this candidate vaccine. The value of antigen P27A should now be challenged in preliminary efficacy trials using a controlled human malaria infection in nonexposed and exposed subjects.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contribution. V. S.-M., K. K., O. K., R. A., C. D., A. F.-M., S. H., L. V., G. C., O. L., S. A., S. Sh., B. G., F. S., and S. A. J. made substantial contributions to the conception and design of the study. V. S.-M., K. K., O. K., S. Ro., R. A., C. D., S. Sh., B. G., F. S., and S. A. J. performed data collection. A. F.-M., A. E.-V., L. V., E. H., and S. Sc. performed data monitoring and reporting. V. S.-M., K. K., O. K., S. Ro., S. H., L. V., S. Sh., B. G., F. S., and S. A. J. performed safety data analysis and interpretation. R. A., A.-C. T., C. M., C. D., C. Mk., D. P., S. Ru., I. F., R. W. T., M. T., G. C., F. S., and S. A. J. performed immunogenicity data analysis and interpretation. V. S.-M., S. Ro., R. A., C. D., S. H., G. C., B. G., F. S., and S. A. J. wrote the manuscript. All authors contributed to the revision of the manuscript and approved the final version. As corresponding author and principal investigator, F. S. had full access to all the data in the study and took responsibility for the decision to submit for publication.

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